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Evaluation of antibacterial activity of *Commiphora myrrha* against antibiotic resistant clinical pathogens

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ABSTRACT

There are many plants used by tribunal people as an anti bacterial, among them myrrh is the one which is commonly used. Myrrh is an oleo gum resin obtained from the plant *Commiphora myrrha* belongs to the family Burceraceae. It can adhere to intestines because of its resinous nature and it reduces the acidity in small intestines. Generally, the resin is collected from bark and stem of the plant by the process incision. In present study ethyl acetate extract of C*ommiphora myrrha* was used for the evaluation of Anti bacterial activity against three Gram negative organisms and two Gram positive organisms. The method used in evaluation of Anti bacterial activity was serial dilution and Agar Diffusion method. The antibacterial activity was calculated in terms of minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC). The antibacterial activity was compared with marketed antibiotic.

Introduction

Myrrh is obtained from *Commiphora myrrha* (family; Burseraceae). Generally, the resin is collected from bark and stem of the plant by the process incision. Myrrh contains 9 to17% volatile oil, 20 to 40% alcohol-soluble resin, and approximately 30 to 60% water-soluble gum[1]. The volatile oil contains heerabolene, acadinene, elemol, eugenol, cuminaldehyde, numerous furanosesquiterpenes including furanodiene, furanodienone, curzerenone, lindestrene.

Mucilage is a thick, glutinous substance related to the natural gums, comprised usually of protein, polysaccharides, and uranides. It also contains gums like xylose, galactose, arabinose, and 4-o methyl glucuronic acid.Some of the chief components in myrrh are sesquiterpenes. Sesquiterpenes are a large family of C15 -isoprenoid molecules found in plants, microbes, and some marine organisms. Isoprenoids also called terpenoids are unsaturated hydrocarbons found in essential oils and oleorsins of plants. Mono terpenoids. Sesquiterpenoids present in volatile fraction and triterpenoids present in nonvolatile fraction.. Along with these it also contains salt, sulphates, benzene, malate and acetate, commiphoric acid, cinnamic aldehyde, furano eudesma 1, 3 diene. Main constituent attribute to its analgesic activity by binding to opoid receptors in brain.It is partly soluble in alcohol, water, and ether. It is soluble in excess of ethyl acetate and dimethyl sulphoxide. Diterpenoids having anti-bacterial activity [2].

Antifungal and anti bacterial activity has been observed in vitro against standard pathogenic strains of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*. The oleo-gum-resin of *C. mukul* was shown to be comparable to kanamycin against both Gram (+) and Gram (-) bacteria in vitro. Also proved to have carminative and anti-inflammatory [3].It is used internally to treat fevers, edema, anemia, constipation, hepatitis, ulcers, skin disorders, obesity, hemorrhoids, cough, asthma, paralysis, gout, and rheumatism [8,9,10]. Anti-inflammatory activity, as observed in an animal model of inflammation. In this study, anti-inflammatory activity was more marked than that of hydrocortisone. An animal model of RA confirmed significant anti-inflammatory effects with oral administration, also resulting in decreased joint swelling [4].

Several compounds found within myrrh exert local anaesthetic activity, chiefly by blocking the inward sodium current across membranes. *Commiphora myrrha* has many medicinal powers and has been used to treat various diseases, such as fever, ache, amenorrhea, tumors, stomach complaints, chest ailments, and skin infections in ancient India [7]. Myrrh has astringent activity, promotes tissue granulation and enhances wound healing [5].

Materials and Methods

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Collection and authentification of plant sample

Commiphora myrrha was collected from local market of Hyderabad authenticated in Pharmacognosy lab of a Arya college of pharmacy. Voucher specimen is deposited in department; with voucher specimen number (ACP-G1). All the chemicals like ethanol ,dimethyl sulfoxide , sulphuric acid are of analytical grade.

The microorganisms were collected from the microbiology laboratory in Hyderabad *viz*. Gram positive organisms – *Streptococcus aureus, Enterococci* and Gram negative organisms - *Escherichia coli* , *Pseudomonas aeruginosa, Klebsiella pnumonae.*

All experiments were carried out by standard published protocols. All glass wares and equipments used for handling of bacterial cultures were sterilized prior to use. All sterilization procedures were performed by autoclaving at 121°C at 15lb pressure.

Methodology

Preparation of extract

The small pieces of oleogum resin of C*ommiphora myrrha* was extracted three times with solvent of ethyl acetate at room temperature for 24hr with mass to volume ratio 1:10 g/ml and was evaporated to dryness under vacuum at 40^{o}C. All extracts were filtered to 0.45um membrane filters. For anti bacterial activity dried residue of extract was dissolved in DMSO (dimethyl sulfoxide

Antibacterial activity of plant extract: The following different growth medias were used to culture the microorganisms.

Media for Bacterial Culture

Composition of Muller Hinton agar medium: peptone-5 grams/litre, beef extract-1.5 grams/litre, yeast-1.5 grams/litre, sodium chloride-5 grams /litre, agar agar-15 grams/litre, PH-7.3 at 25°C suspended 35 gm in 1000 ml of water.

Composition of Nutrient broth: Enzymatic digested gelatine -8 gm, beef extract-5 gm, final PH 25°C suspended 13 gm in 1000 ml of water.

Preparation of Media: Accurately weighed the Muller Hinton agar medium, transferred in to clean conical flask and volume made up to 200ml with continuous shaking. Heated to dissolve the media in the distilled water. The conical flask was tightly closed with cotton plug and autoclaved at 121°C at 15Lb pressure.

Preparation of Nutrient Broath: Accurately weighed 13gm of nutrient broth and transferred in to clean conical flask and made the volume up to 1000 ml with continuous shaking. prepared broth was dispensed in to glass tubes each 10 ml and sealed tightly with cotton plugs. Tubes were autoclaved at 121°C for 20 minutes at 15Lb pressure.

Preparation of culture: All bacterial strains were obtained from microbial type culture collection. Different bacterial strains were maintained on nutrient agar and subcultures were freshly prepared before use.

Bacterial cultures were prepared by two methods

1. Plate Preparation: Using sterile conditions 20 ml of agar medium was transferred into sterile petri dishes. After solidifying, the plates were used for the assay.

2. Sub-Culturing: Subcultures were prepared by transferring loop full of inoculum from culture slants to freshly prepared agar slants. These were incubated in the desired conditions.

Determination of Antibacterial Activity

The Broth Dilution Method

Preparation of Microorganisms

The strains were maintained on sterilized Mueller-Hinton agar slants at 4°C. A loop full of each bacterial strain from agar slants was inoculated into 50 ml of sterile Nutrient broth in 100 ml conical flask which was sterilized at 121°C for 20 min at 15Lb pressure. The flasks were incubated at 37°C for 4-6hrs to activate the strain. Flasks containing the bacterial strain in broth were kept at 4°C for storage.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC)

MIC and MBC of extracts were determined by tube broth dilution assay. A sterile 2-fold dilution, ranging from 50mg - 0.39 mg/ml of extract prepared and 500 ul of each dilution was incubated with 2.47 ml of nutrient broth containing 30 µl of inoculum at 37°C for 24hrs. The MIC was determined at lowest concentration that demonstrates no visible growth by macroscopic evaluation. After determination of MIC, tubes showing no turbidity were diluted 100-fold with drug free nutrient broth and incubated at 37°C for 48 hrs. The lowest concentration of tube that showed no visible growth in drug free cultivation was considered as the MBC.

Determination of Antibacterial Activity by Agar Well Diffusion Method [6]

An agar-well diffusion method was employed for evaluation of antibacterial activity. The bacterial strains were reactivated from stock cultures by transferring into nutrient broth and incubating at 37°C for 18 hrs. A final inoculums containing 10^6 colony forming units ($1x10^6$ CFU/ml) was added to wells (8 mm in diameter) punched on agar surface. Plates were incubated overnight at 37°C and diameter of inhibition zone (DIZ) around each well was measured in mm. Antibiotics at the concentration of 100ug/well were used as positive reference to determine sensitivity of microorganisms tested.

Results and Discussion

Antibacterial activity by dilution method: The antibacterial activity of ethyl acetate extract of *C. myrrha* showed in Ttable-1. the MIC and MBC was calculated by observing growth and turbidity of nutrient broth.

Table 1: Determination of MIC and MBC of plant extract against different microorganisms (mg/ml)

Organisms	MIC(mg/ml)	MBC(mg/ml)
Escherichia coli	12.5	25
Enterococcus	3.12	12.5
Pseudomonas aeruginosa	6.25	12.5
Klebsiella pneumonae	6.25	12.5
Streptococcus aureus	6.25	12.5

The results obtained in the present study showed that MIC against *Enterococci* is high i.e. 3.12mg/ml.

Agar well diffusion method: The antibacterial activity was done by using different concentrations of *C. myrrha* extract on different organisms shoed in table-2. Zone of inhibition was measured in mm.

Table 2: Agar well diffusion of plant extract against the different micro organisms (mg/ml)

Organism	100 mg/ml	50 mg/ml	25 mg/ml
Escherichia coli	10 mm	6 mm	0
Enterococcus	17 mm	12 mm	10 mm
Pseudomonas aeruginosa	11 mm	10 mm	0
Klebsiella pneumonae	10 mm	0	0
Streptococcus aureus	9 mm	5 mm	0

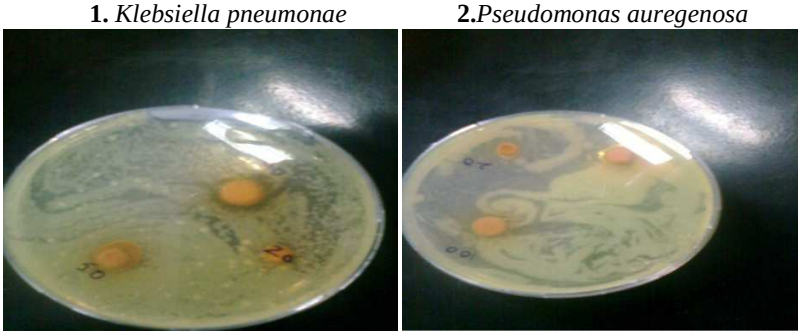
Five bacterial strains were used to evaluate the effect of plant extract by agar well diffusion method. In all the organisms the highest zone of inhibition was observed for *Enterococci* at 100 mg/ml (17 mm). And no inhibition zone found for *Klebsiella pneumonae* at 50mg and 25mg.

Finally the plant extract shows the inhibition zones at 100mg, 50mg, 25mg, against the organism *Enterococci*. For remaining organisms less or no inhibition zones were found. So *Enterococci* organism is more sensitive to *Commiphora myrrha.*

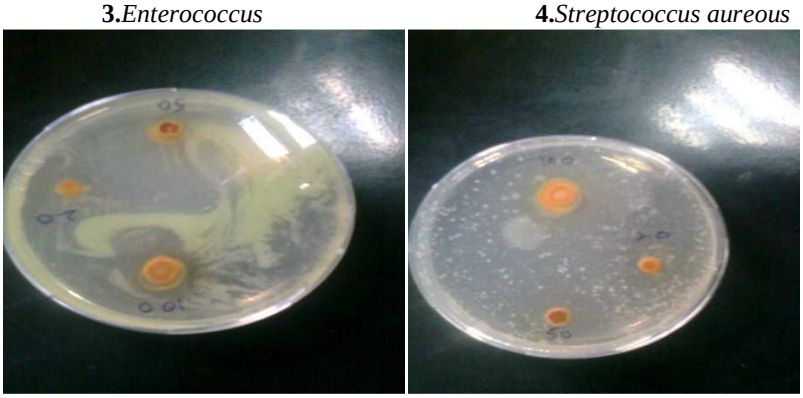
The zone of inhibition obtained for different organisms is presented in Figure-1.

Figures 1: Agar well diffusion method of plant extract against the different microorganisms (mg/ml)

1. *Klebsiella pneumonae* **2.***Pseudomonas auregenosa*



3.*Enterococcus* **4.***Streptococcus aureous*



5. *Escherichia coli*

Comparison study of antibacterial activity of plant extract with antibiotic on clinical pathogens by Agar well diffusion method

The Comparison study of antibacterial activity of *C. myrrha* with marketed antibiotic was performed on different organisms and showed in table-3. The zone of inhibition obtained for different organisms is presented in figure-2.

Table 3: Comparison study of antibacterial activity of plant extract with antibiotic on clinical pathogens

Organisms	Extract (200mg/ml)	Antibiotic (100mg/ml)
Klebsiella pneumonae	15 mm	26 mm
Streptococcus aureoous	10 mm	25 mm
Enterococcus	22 mm	30 mm
Escherichia coli	17 mm	24 mm
Pseudomonas aeruginosa	12 mm	40 mm

Agar well diffusion method of cefataxime sodium, a cephalosporin antibiotic shown the highest zone of inhibition against *Pseudomonas aeruginosa* at 100 mg/ml (40 mm) where as *Commiphora myrrha* extract shows the highest zone of inhibition against *Enterococci* at 200 mg/ml (22mm).

Figures 2 : Comparison of antibacterial activity of c.myrrha with marketed antibiotic on clinical pathogens by Agar well diffusion method

1.*Streptococcus aureous*

2 . *Klebsiella pneumonae*

3*. E.coli*

4. Enterococcus

5. *Pseudomonas auregenosa*

Conclusion

In this study *Commiphora myrrha* was extracted and its biological activity was tested against antibacterial resistant organisms.The study shows that the organism *Enterococci* shows high sensitivity to *Commiphora myrrha* plant extract.The minimum inhibitory concentration (MIC) and MBC according to tube dilution method was found to be 3.12mg/ml and 12.5mg/ml respectively.

Further the antibacterial activity of extract was compared with antibiotic like cefataxime against different organisms. The extract shows the high antibacterial activity against *Enterococci* organism where as cefataxime shows the high antibacterial activity against *Pseudomonas aureginosa.* The extract shows high zone of inhibition against *Enterococci* (17mm) by agar well diffusion method.

Many herbs are being used by the tribunal people. The scientific data should be produced to explore the activities of these herbal drug. In this research a small attempt has been made to explore the activity of *C.myrrha*, such kinds of study will surely help to explore the claims of herbal drugs.

Conflict of interest: We declare that we have no conflict of interest.

References

1. C.K.Kokate, A.P. Purohit, S.B. Gokhale; Pharmacognosy, Nirali Prakashan, 50th edition, 2014; 14.140.
2. Al-Harbi, M.M., S. Qureshi, M.M. Ahmed, S. Rafatullah and A.H. Shah,. Effect of *Commiphora molmol* (aloe-gum-resin) on cytological and chemical changes induced by cyclophosphamide in mice. Am. J. Chinese Med. 1994; 32: 77-82.
3. Tarig, M., M.A. Al-Yahya, J.S. Mossa, I.A. Al-Meshal and A.A. Al-Badr, Phytochemical, pharmacognostical and pharmacological studies on CNS depressant plants of Saudi Arabia,Proceedings the 4th International Congress of Pharmaceutical Science, (ICPS`85), Montreal, Canada,1985, 122-122.
4. Provan, G.J. and P.G. Waterman,. Major triterpenes from the resins of *Commiphora incise*, Phytochemistry, 1988; 27: 3841-3843
5. Reynolds, J.E.F., Martindale-The Extra Pharmacopoeia. 31st Edn., Royal Pharmaceutical Society, London,1996.
6. Shan B, Cai YZ, Brooks JD, Corke H. The *in vitro* antibacterial activity of dietary spice and medicinal herb extracts. Int J Food Microbiol, 2007; 117:112–9.
7. Shen T, Lou Hx. Chemical constituents from resin of *Commiphora* species and their biological activities, Nat Prod Res Dev, 2008; 20:360–6.
8. Anonymous, New Delhi: Department of Indian Systems of Medicine and Homeopathy; 2001. The Ayurvedic Pharmacopoeia of India.
9. Anonymous. New Delhi: Council of Scientific and Industrial Research; 2007. The Wealth of India: Raw Materials.
10. Kohli KR, Nipanikar SU, Kadbhane KP. A comprehensive review on *Trivrit Operculina turpethum*syn. *Ipomoea turpethum*. Int J Pharma Bio Sci. 2010;1:443–52.

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